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Final Report

Economic Benefits to Early Calfhood Vaccination Against Respiratory Diseases

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a) Executive Summary

1) Abstract

This study was undertaken to determine if vaccination can enhance immune responses in pregnant cows and their young calves. . The results of this study indicate that cows immunized in the late stages of pregnancy with a vaccine containing modified-live viruses can respond to the vaccine with enhanced antibody and cellular immune responses compared to unvaccinated penmates. There was enhanced passive immunity to bovine respiratory syncytial virus in calves from vaccinated dams. Although calves vaccinated in the first two weeks of life did not they did have significantly higher virus-specific cellular immune responses than unvaccinated penmates. These findings indicate that the cellular immune system of young calves can be primed by vaccination despite the presence of circulating maternal antibodies.

2) Summary

A study was undertaken to determine the immunological effects of vaccination of pregnant cows and their young calves. Fifty two cows were randomly assigned to two groups. One group of 26 cows was vaccinated twice with a vaccine containing modified-live viruses approximately 28 and 14 days prior to calving. The other group of 26 cows served as unvaccinated commingled controls. Thirty three calves that were born within approximately 2 weeks were used as principals in the first part of this study; thirteen calves from vaccinated dams and 12 from unvaccinated dams. An additional 8 calves from unvaccinated dams served as unvaccinated commingled controls. Sera were collected from cows on the day of vaccination and at calving. Colostrum was collected within 24 hours of calving. Antibodies and cellular immune responses to bovine respiratory syncytial virus (BRSV) and and bovine herpes virus-1 (BHV-1) were measured in blood and colostrum. In addition, skin tests were used to determine if there was transfer of BRSV-specific cells in the milk. Cellular responses and antibody production were again assessed in response to vaccination at weaning in a group of 16 neonatally vaccinated calves and an additional 15 unvaccinated penmates.

On the basis of antibody tests, all the multiparous cows used in this study had serological evidence of previous exposure to BRSV and BHV-1 and there were no significant differences between the groups prior to immunization. At parturition there were significant increases in serum antibodies to BRSV and BHV-1 in immunized cows. There were no significant differences in the amounts of BRSV-specific or BHV-1 specific antibodies in the colostrum between groups of cows. Calves from

immunized cows had significantly higher concentrations of BRSV-specific serum antibodies on days 10 and 22 of life compared to commingled calves from unimmunized dams; however, there were no significant differences in serum antibodies specific for BHV-1 between the two groups of calves. There were no differences in the concentrations of virus-specific antibodies either prior to or after vaccination at weaning between groups of neonatally vaccinated and unvaccinated calves.

Blood lymphocytes from vaccinated cows responded better to BRSV than cells from their non-immunized penmates. White blood cell populations from both groups of cows generally contained a high percentage of macrophages and responded poorly to BRSV antigens. Although white blood cells from young calves had low or suppressed responses to live BRSV at 10 days of age, immunization at this time induced significant increases in cellular immune responses to BRSV and BHV. Prior to vaccination at weaning, white blood cells from neonatally vaccinated calves had significantly higher levels virus-specific cellular immune responses than unvaccinated penmates. There were no differences between groups after vaccination at weaning. White blood cells from most vaccinated and unvaccinated calves produced interferon gamma upon exposure to BHV-1 or BRSV in culture and there were no differences among treatment groups.

Our results indicate that although there were usually no measurable increases in antibody responses to modified-live viruses following vaccination of young calves there were virus-specific cellular immune responses most vaccinated calves. These virus-specific memory responses persisted in most calves until weaning. These findings suggest that the cellular immune system of young calves with maternal antibodies can be primed by immunization with modified-live viruses during the neonatal period. Furthermore, vaccination of cows in the late stages of pregnancy may enhance passive transfer of immunity to young calves.

b) Technical Report

Abstract

A study was conducted in two phases to determine the effect of perinatal vaccination on immune responses in cows and calves. Fifty two cows were randomly assigned to two groups. One group of 26 cows was vaccinated twice with a polyvalent vaccine approximately 28 and 14 days prior to calving. The other group of 26 cows served as unvaccinated commingled controls. Thirty three calves that were born within approximately 2 weeks were used as principals

in the first part of this study; thirteen calves from vaccinated dams and 12 from unvaccinated dams. An additional 8 calves from unvaccinated dams served as unvaccinated commingled controls. Sera were collected from cows on the day of vaccination and at calving. Colostrum was collected within 24 hours of calving. Antibodies and lymphocyte proliferative responses to BRSV and BHV-1 were measured in blood and colostrum. In addition, calves were assessed for passive transfer of lymphocytes that mediate delayed type hypersensitivity (DTH) responses to BRSV at 8 days of age prior to immunization. In phase II of the study, lymphocyte proliferative responses and antibody production were again assessed in response to vaccination at weaning in a group of 16 neonatally vaccinated calves and an additional 15 unvaccinated penmates.

On the basis of enzyme-linked immunosorbent assays, all the multiparous cows used in this study had serological evidence of previous exposure to BRSV and BHV-1 and there were no significant differences between the groups prior to immunization. At parturition there were significant increases in serum antibodies to BRSV and BHV-1 in immunized cows. There were no significant differences in the amounts of BRSV-specific or BHV-1 specific antibodies in the colostrum between groups of cows. Calves from immunized cows had significantly higher concentrations of BRSV-specific serum antibodies on days 10 and 22 of life compared to commingled calves from unimmunized dams; however, there were no significant differences in serum antibodies specific for BHV-1 between the two groups of calves. There were no differences in the concentrations of virus-specific antibodies either prior to or after vaccination at weaning between groups of neonatally vaccinated and unvaccinated calves.

Blood lymphocytes from vaccinated cows had significantly higher blastogenic responses to BRSV than cells from their non-immunized penmates. Colostral leukocyte populations from both groups of cows generally contained a high percentage of macrophages and had poor proliferative responses to BRSV antigens. Although blood leukocytes from young calves had low or suppressed responses to live BRSV at 10 days of age, immunization at this time induced significant increases in blastogenic responses to BRSV and BHV. Prior to vaccination at weaning, lymphocytes from neonatally vaccinated calves had significantly higher levels virus-specific proliferation than unvaccinated penmates. There were no differences between groups after vaccination at weaning. Leukocytes from most vaccinated and unvaccinated calves produced interferon gamma upon exposure to BHV-1 or BRSV in vitro and there were no differences among treatment groups.

Our results indicate that although there were usually no measurable increases in humoral responses to modified-live viruses following vaccination of young calves there was virus-specific proliferation of lymphocytes from most vaccinated calves. Virus-specific memory cells persisted in most calves until weaning. These findings suggest that the cellular immune system of young calves

Experimental Methods

1. Experimental Design

This study examined the development of immune responses to BRSV, BHV-1 in cow/calf pairs from the University of Saskatchewan's beef herd at Goodale Farm where there is currently not routine vaccination for BRSV In order ensure that 25 calves would be born over a period of approximately two weeks, fifty-two cows were randomly assigned to two groups. One group were vaccinated twice with a modified-live polyvalent vaccine at approximately 28 and 14 days prior to calving. The other group of 26 cows served as unvaccinated commingled controls. Twenty-five calves were vaccinated with a modified-live polyvalent vaccine on day 10 after birth. Another group of 8 unvaccinated calves from dams that were not vaccinated served as controls.

Sera were collected from the cows on day 0 (day of vaccination) and at calving. Samples were aliquoted, frozen and stored. Antibodies to BRSV and BHV-1 were assessed at the Western College of Veterinary Medicine using ELISA. Colostrum was collected from the cows within 24 hr of calving. Antibodies and lymphocyte blastogenic responses to BRSV and BHV-1 were assessed at the Western College of Veterinary Medicine. Antibody responses to BRSV and BHV-1 were assessed in calves using sera collected on the day of vaccination and 12 days after vaccination. Cellular immune responses to BRSV to BHV-1 were assessed in the peripheral blood of cows and calves, and in colostrum. All assays were conducted in blind fashion.

2. Methodology

Enzyme-Linked Immunosorbent Assays (ELISA) for BRSV and BHV-1- ELISAs for BRSV and BHV-1 antibodies utilized partially purified viruses derived from lysed bovine tracheal cells infected with the BRSV or BHV-1 and was performed according to previously described methods (Durham and Hassard, 1990) using duplicate serum samples diluted 1/50. Lysed uninfected bovine tracheal cells were used as control antigen. The optical density values were converted to units that were determined by comparison of raw optical density values with a standard curve that was generated by linear regression of results obtained with serial dilutions of BRSV and BHV-1 hyperimmune serum from a steer. If the antibody concentration was approximated as a negative value from the regression line, then the concentration was considered to be 0.

Virus neutralization (VN) assays- Virus neutralizing antibodies specific for BRSV in serum were measured using microtiter plaque reduction assay that was performed essentially according to previously described methods (Ellis et al., 1990; Durham et al., 1991) using the 375 isolate of BRSV Samples were tested in duplicate and serum was diluted using 3-fold serial dilutions (1:3-1:81). Samples were further diluted 1:1 with cultured virus so that the reciprocal dilutions tested were 6, 18, 54, and 162. Titers were recorded as the last dilution

that yielded positive results (neutralization of infectivity) in both replicates. If only one replicate was positive, it was considered to have one-half titer lower concentration. For the purpose of analyses, VN reciprocal titers were coded; titers recorded as <6, 6, 12, 18, 36, 54, 108, 162, >162, were assigned codes of 0, 1, 2, 3, 4, 5, 6, 7, or 8 respectively (Thursfield, 1986)

Lymphocyte Blastogenesis Assays- Jugular blood was collected from vaccinated and unvaccinated cows at the time of calving, and from calves prior to vaccination on day 9 or 10 of life and on day 12 after vaccination. Mononuclear leukocytes were separated from the blood and colostrum using density gradient centrifugation (Ellis et al., 1992), with Ficoll/Hypaque of specific gravities 1.077 and 1.083, respectively. Lymphocyte blastogenesis assays were performed according to previously described methods (Ellis et al.,1992) using a standardized dilution of live BRSV 375 isolate that was grown in bovine kidney (BK-6) cells with minimal serum (stock culture approximately $10^{6.5-7}$ tissue culture infective dose $(TCID)_{50}$/ml) or BHV-1 that was grown in Maden-Darby bovine kidney (MDBK) cells (stock culture approximately 10^{8} tissue culture infective dose $(TCID)_{50}$/ml) and subsequently UV-inactivated and heat treated (56 C for 30 minutes). Lysates of uninfected BK-6 or MDBK cells were the tissue culture controls in the blastogenesis assays for BRSV and BHV-1, respectively. Standardized dilutions of antigens were determined using leukocytes from two adult dairy cattle that were immunized twice with both a modified-live polyvalent vaccine and an inactivated vaccine. Results are reported delta counts per minute (cpm) that were determined as follows: [mean cpm in wells cultured with BK-6 cells or MDBK cells infected with BRSV or BHV-1] - [mean cpm in wells cultured with uninfected BK-6 cells or MDBK] and were used to compare groups of vaccinees at different time points and among treatment groups following log transformation (Hughes et al., 1984)

Phenotyping of Colostral Leukocytes- Cytospins were prepared from colostral leukocyte suspensions that were obtained by density gradient centrifugation. Leukocytes were reacted with monoclonal antibodies specific for BoCD5, BoCD4, BoCD8 and a monocyte/macrophage-specific antigen (ILA-15) using an indirect immunoperoxidase method as previously described (Ellis et al., 1992). Colostral leukocytes from 6 cows were examined.

ELISA for Interferon gamma (IFN-γ) - An ELISA for bovine IFN-γ was performed using bovine IFN-γ-specific monoclonal and monospecific polyclonal antibodies as capture and detection reagents, respectively. IFN-γ was quantitated in supernatants from leukocyte cultures that were stimulated with BRSV, BHV-1 or Con A and incubated for 60 hr. The concentration of IFN-γ in supernatants was determined by comparing optical density values in test wells with a standard curve that was derived by regression analysis using OD values from analysis of serial dilutions of recombinant bovine IFN-γ. Concentrations of virus-specific

interferon were calculated as follows: (concentration of IFN in virus-stimulated cultures) - (concentration of IFN in cultures with uninfected cell lysates).

Delayed Type Hypersensitivity (DTH) Reactions - As a further *in vivo* assessment of the passive transfer of cellular immunity to BRSV, cutaneous DTH tests similar to those used in the assessment of tuberculin responses were performed. This was accomplished by injecting 100 µl of BK cell lysate or BRSV-infected BK lysate intradermally in shaved skin of the neck of calves at 1 week of age. Skin thickness will be measured in both sites with a caliper 48 hr later.

Statistical Analyses- Responses in the treatment groups were analyzed for the antibody and IFN-γ concentrations and delta counts per minute values (following log transformation) for lymphocyte blastogenesis tests. Critical alpha level for significance of statistical tests was set at p=0.05. Significance of change in status between (2 groups) and within a treatment group was tested using unpaired and paired Students *t* -tests, respectively. Differences in responses among treatment groups were evaluated using analysis of variance (ANOVA). Least squares linear regression was used to determine if levels of serum antibody were associated with levels of colostral antibodies. The colostral antibody levels were used as dependent variable in separate analyses with serum antibody levels the independent variable. Analyses were performed using Statworksr software (Cricket Software, Philadelphia, PA).

Results

Clinical responses to vaccination-No adverse effects were noted in cows that received intramuscular injections with a modified-live polyvalent vaccine.

BRSV-Specific Antibody Responses in Cows (Table 1)- On the basis of ELISA all the multiparous cows used in this study had serological evidence of previous expo-sure to BRSV (ELISA units>20) prior to vaccination with a modified-live polyvalent vaccine, and there were no significant differences between groups in serum concentrations of BRSV-specific antibodies prior to inoculation. There were no significant increases in BRSV-specific antibodies when sera were compared before and after two immunizations with a modified-live polyvalent vaccine, as measured by ELISA. Neither were there any significant changes in BRSV-specific antibodies in the commingled control cows that were not immunized. There were, however significant increases in VN antibodies in immunized cows. Although colostrum from all cows tested contained BRSV-specific antibodies, there were no significant differences between vaccinated and unvaccinated cows, as measured by ELISA. Approximately 28% of the variation in the levels of BRSV-specific colostral antibody was predicted by variation in post partum serum concentrations of BRSV-specific antibody as determined by ELISA (p=0.003; R_2=0.273).

BRSV-Specific Antibody Responses in Calves (Table 2) Although there were no significant differences in BRSV-specific antibodies in the milk of

vaccinated and unvaccinated cows, calves from vaccinated cows had significantly higher concentrations of BRSV-specific antibodies in there serum on day 10 ($p=0.008$) and day 22 ($p=0.038$) of life.

BHV-1-Specific Antibody Responses in Cows-(Table 1) All cows used in this study were vaccinated in the fall of 1992 with a modified-live BHV-1 vaccine and on the basis of ELISA all these multiparous cows had antibody responses to BHV-1 (ELISA units>20) prior to vaccination with a modified-live polyvalent vaccine (Table 3). There were no significant differences between groups in serum concentrations of BRSV-specific antibodies prior to inoculation with a modified-live polyvalent vaccine. There were significant ($p<0.001$) increases in BHV-1-specific antibodies in cows that received 2 immunizations with a modified-live polyvalent vaccine when sera were compared prior to inoculation. There were no significant changes in BHV-1-specific antibodies in the commingled control cows that were not immunized. Although colostrum from all cows tested contained BHV-1-specific antibodies, there were no significant differences between vaccinated and unvaccinated cows. Approximately 35% of the variation in the levels of BHV-1-specific colostral antibody was predicted by variation in post partum serum concentrations of BHV-1-specific antibody as determined by ELISA ($p<0.0001$; $R_2=0.354$).

BHV-1-Specific Antibody Responses in Calves (Table 2)-All calves tested had evidence of passive transfer of BHV-1 specific antibodies; however, there were no significant differences in BHV-1-specific antibodies in the serum of calves from vaccinated or unvaccinated cows on day 10 or day 22 of life.

BRSV-Specific Lymphocyte proliferative Responses in Cows (Table 1)- Cows that were immunized twice with *a modified-live polyvalent vaccine* in the late stages of pregnancy had significantly ($p=0.013$) higher blastogenic to BRSV than unvaccinated penmates when peripheral blood leukocytes were harvested post partum.

Cellular responses in colostrum (Table 1)- In 5 of 6 randomly sampled colostrum samples ILA-15+ macrophages were the predominate population and accounted for 50-90% of cells, as determined on the basis of morphological and immunohistochemical criteria. CD5+ were a minority population in colostral samples examined and comprised approximately equal numbers of CD5+/CD4+ and CD5+/CD8+ lymphocytes. Probably because of the high numbers of macrophages, colostral leukocyte populations had poor proliferative responses to BRSV antigens.

Lymphocyte Proliferative Responses to BRSV, BHV-1, or Mitogen in Calves (Table 2)- Leukocytes from 10-day old calves harvested prior to immunization had poor and often suppressed responses to BRSV, irrespective of the vaccination status of the dam. Leukocytes from most 22-day old calves harvested 12 days after one immunization with a modified-live polyvalent vaccine responded to BRSV and BHV-1. The responses BRSV were significantly

($p<0.001$) higher than responses on day 10. There were significantly higher proliferative responses to BRSV ($p=0.015$) and BHV-1 ($p=0.006$) in vaccinated calves compared to responses in a group of age-matched unvaccinated calves from unvaccinated dams. There were no significant differences in lymphocyte proliferative responses to BRSV or BHV-1 between groups of vaccinated calves from vaccinated and unvaccinated dams. Leukocytes from 22-day old calves had variable proliferative responses to concanavalin-A. There were no significant differences in proliferative responses between groups of vaccinated calves from vaccinated and unvaccinated dams and unvaccinated calves.

Interferon-Gamma (IFN-γ) Responses to BRSV, BHV or Mitogen in Calves (Table 2)- There was IFN-γ in the supernatants from leukocyte cultures stimulated with BRSV or BHV-1 from most calves tested. Levels of this cytokine produced in response to these antigens were markedly lower than those produced in response to the mitogen concanavalin-A. In contrast to lymphocyte blastogenic responses, there were no significant differences between groups of vaccinated and unvaccinated calves (Table 2).

Delayed - Type Hypersensitivity (DTH) Reactions to BRSV in Calves - No palpable cutaneous DTH reactions were observed following intradermal challenge of 9 day old calves with modified live BRSV.

Discussion

1) Immunization of BRSV-seropositive cattle with a modified-live polyvalent vaccine in the late stages of pregnancy did not result in significant increases in BRSV-specific antibodies in the serum compared to levels in unvaccinated penmates, as measured by ELISA. There were, however, increases in serum neutralizing antibodies. These results are consistent with previous findings indicating a disparity between ELISA and VN tests for BRSV (Ellis et al., in press). There were no significant differences between BRSV-specific antibodies in the colostrum of vaccinated and unvaccinated cows. This may be in part attributable to the level of BRSV-specific antibodies in the serum of the dams or to differences in colostrum samples, i.e. differences in the relative concentrations of antibodies in milk from quarters with subclinical inflammation or collection of milk from quarters that had been suckled. However, calves from dams vaccinated in the late stages of pregnancy did have significantly higher levels of BRSV-specific antibodies than calves from previously exposed dams that were not vaccinated in the late stages of pregnancy.

2) In contrast to BRSV, immunization of cows with a modified-live polyvalent vaccine in the late stages of pregnancy resulted in significant increases BHV-1-specific serum antibodies. This enhanced response to BHV-1 was not seen in increased colostral levels of antibodies to BHV-1 (compared to unvaccinated controls), nor in increased levels of BHV-1-specific antibodies in the sera of calves from vaccinated dams. The reasons for the apparent disparity in these antibody responses to BRSV versus BHV-1 in cows and calves, are not apparent

but may be related to the fact that these cows were vaccinated earlier in pregnancy with modified-live BHV.

3) The fact that immunization of young with a modified-live polyvalent vaccine on day 10 of life did not result in the production of increased serum levels of antibodies to BRSV or BHV-1 is compatible with the current dogma regarding the blocking effect of maternal antibodies on antibody response to modified-live viral vaccines.

4) Although antibody responses to BRSV and BHV-1 were not dramatic in cows or calves, lymphocyte proliferative responses to BRSV were significantly greater in vaccinated cows and calves compared to unvaccinated penmates. The ability of perinatal immunization to stimulate virus-specific T lymphocyte responses in young calves suggests that *in vivo* priming of the cellular immune system can take place despite the presence of maternal antibodies. This data provides cellular evidence for priming of the immune system as has been previously suggested by Kimman et al. (1989). Moreover, the ability of young cattle to respond to viral antigens with IFN-γ production is a further indication of the stimulation of cellular immunity in the face of maternal antibodies.

5) The investigation of virus specific lymphocyte responses in colostrum was complicated by the high proportion of apparently suppressive macrophages in these samples. Future studies employing plastic adsorption of macrophages and subsequent assay of residual lymphocytes are planned.

6) These data were unable to document passive transfer of cellular immunity to calves in measurable by the of presence of virus-specific lymphocytes in colostrum nor by evidence of passive transfer of lymphocytes mediating BRSV-specific DTH responses. More sensitive methods are apparently necessary to detect the low frequency of maternal virus-specific T lymphocytes that are probably transferred to the neonate.

Conclusions and Impact

In conclusion, the major finding of this phase of the study was documentation of stimulation of virus-specific T lymphocytes following perinatal inoculation of young calves with a modified-live polyvalent vaccine. These CMI responses occurred in the presence of maternal antibody and provide evidence of T cell priming. Furthermore, our results indicate that cows can be immunized with in the late stages of pregnancy with this modified-live viral vaccine, and that this can enhance passive transfer of immunoglobulins to the calves. This effect is likely to be most pronounced in cows with low levels of immunity prior to immunization. Although there was no respiratory disease in this group of calves, The impact of these findings should be a reevaluation of the use of modified-live viral vaccines in young calves. Such usage would likely have economic benefits if respiratory disease was a problem at a particular facility. Moreover immunization of cows in the latter stages of pregnancy may yield enhanced passive immunity by means of increasing colostral antibodies.

Table 1. Immune responses in cows

Test/Time	Unvaccinated Cows	Vaccinated Cows
BRSV ELISA[1] day 0	76.5 ± 3.2	84.6 ± 5.4
BRSV ELISA / pp[2]	70.0 ± 4.0	84.7 ± 4.8
BRSV SN[3] / day 0	5.4 ± 0.4	5.7 ± 0.4
BRSV SN[1] / pp	5.7 ± 0.4	7.2 ± 0.2*
BHV-1 ELISA / serum / day 0	58.8 ± 5.2	63.6 ± 7.3
BHV-1 ELISA / serum / pp	54.4 ± 4.4	81.3 ± 24.6*
BRSV ELISA / colostrum	66.0 ± 6.0	65.0 ± 6.0
BHV-1 ELISA / colostrum	59.0 ± 7.4	60.2 ± 1.2
BRSV LBT[4] / pp	6.2 ± 1.0	8.9 ± 0.3*

[1] Enzyme-linked immunosorbant assay. Data are presented as group mean ELISA units ± standard error of the mean.

[2]post partum

[3] Serum neutralizing antibodies. Data are presented as geometric mean titer ± standard error of the mean.

[4] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

* indicates significant ($p<0.05$) difference between groups

Table 2. Immune responses in neonatal calves

Test/Time	Unvaccinated Calves (Unvaccinated Cows)	Vaccinated Calves (Unvaccinated Cows)	Vaccinated Calves (Vaccinated Cows)
BRSV ELISA[1] / day 10	ND[2]	74.8 ± 4.1	94.8 ± 5.6*
BRSV ELISA / day 22	ND	72.5 ± 4.7	88.0 ± 4.6*
BHV-1 ELISA / day 10	ND	60.2 ± 5.8	61.9 ± 7.4
BHV-1 ELISA / day 22	ND	45.3 ± 4.9	53.1 ± 6.5
BRSV LBT[3] / day 10	ND	2.5 ± 0.7	1.3 ± 0.7
BRSV LBT / day 22	0.7 ± 0.7*	5.8 ± 1.2	5.0 ± 1.1
BHV-1 LBT / day 22	0.8 ± 0.8*	5.9 ± 1.2	5.8 ± 1.1
Con A[4] LBT / day 22	11.5 ± 0.1	10.8 ± 0.2	11.3 ± 0.1
BRSV IFNγ[5] / day 22	107.0 ± 56.0	77.0 ± 37.2	32.5 ± 13.0
BHV-1 IFNγ / day 22	132.0 ± 49.0	108.0 ± 49.0	62.0 ± 18.0
Con A IFNγ / day 22	1430.0 ± 337.0	732.0 ± 233.0	1150.0 ± 277.0

[1] Enzyme-linked immunosorbant assay. Data are presented as group mean ELISA units ± standard error of the mean.

[2] Not done

[3] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

[4] Concanavalin A (phytomitogen)

[5] Interferon gamma. Quantitated with ELISA. Data are presented as group mean picograms / ml ± standard error of the mean.

* indicates significant ($p<0.05$) difference between groups

Phase II

Background and Objectives

In phase I of the study we demonstrated that immunization of young calves with modified-live virus vaccine resulted in significant increases in lymphocyte blastogenic responses to BRSV and BHV-1 compared with unvaccinated calves. This cellular immune response occurred in the face of high levels of passively transferred maternal antibody and without appreciable changes in the virus specific antibody status in the young animals following calfhood immunization. These data provide evidence that the immune system of calves can be primed by neonatal immunization without apparent blocking effects by maternal antibody. Furthermore, these data provide cellular evidence of a mechanism of priming for enhanced mucosal responses in calves with maternal antibody as documented by Kimman et al. (1989)

The next relevant question to ask was, "do calves that were immunologically primed in the neonatal period respond significantly better to vaccination at weaning time when, traditionally, they would receive their first immunizations?"

The overall objective of this phase of the study was to determine if calves that were immunized perinatally with modified-live virus vaccine had enhanced responses BRSV and BHV-1 following immunization at weaning. Pursuant to this the following objectives were addressed:

1) Determine levels of serum neutralizing antibodies to BRSV and BHV-1 following immunization of previously vaccinated and unvaccinated calves at weaning.

2) Determine lymphocyte blastogenic responses to BRSV and BHV-1 following immunization of previously vaccinated and unvaccinated calves at weaning.

3) Determine levels of BRSV and BHV-1-induced interferon-γ in leukocyte cultures following immunization of previously vaccinated and unvaccinated calves at weaning.

Experimental Methods

1. Experimental Design

This study examined the immune responses in 2 groups of calves (15 and 16, respectively) that were the subjects of the previous study on perinatal immunization that was conducted in the Spring of 1993.

Calves were from the University of Saskatchewan's beef herd at Goodale farm. Calves were weaned on the day before vaccination (approximately 6 months of age; 400-500 pounds), were kept in two pens, and were separated from other cattle throughout the course of the study. One group of 16 calves had been immunized with both modified-live virus vaccine and a Pasteurella bacterin on day 9 or 10 of life; the other group of 15 calves had not received perinatal

immunizations and were commingled controls. All calves were vaccinated with both modified-live virus vaccine and a *Pasteurella* bacterin at weaning.

Serum was collected from the calves on day 0 (day of vaccination) and on day 12 after immunization. Samples were aliquoted, frozen and stored. Antibodies to BRSV and BHV-1 were assessed using serum neutralization.

Cellular immune responses were assessed in the peripheral blood of calves. Jugular blood were collected prior to vaccination on day 0 and on day 12 after vaccination. All assays were conducted in blind fashion.

2. *Methodology*

Virus neutralization (VN) assays- Virus neutralizing antibodies in serum were measured using microtiter plaque reduction assay that was performed essentially according to previously described methods (Ellis et al., 1990; Durham et al., 1991) using the 375 isolate of BRSV and a field isolate of BHV-1. Samples were tested in duplicate and serum was diluted using 3-fold serial dilutions (1:3-1:81). Samples were further diluted 1:1 with cultured virus so that the reciprocal dilutions tested were 6, 18, 54, and 162. Titers were recorded as the last dilution that yielded positive results (neutralization of infectivity) in both replicates. If only one replicate was positive, it was considered to have one-half titer lower concentration. For the purpose of analyses, VN reciprocal titers were coded; titers recorded as <6, 6, 12, 18, 36, 54, 108, 162, >162, were assigned codes of 0, 1, 2, 3, 4, 5, 6, 7, or 8 respectively (Thursfield, 1986)

Lymphocyte blastogenesis tests- Virus- specific lymphocyte blastogenesis tests (LBT) were performed according to previously described methods (Ellis et al., 1992) using standardized dilutions (1/200) of live BRSV 375 isolate and UV-inactivated, heat inactivated (30 minutes at 50 C) that were grown in bovine kidney (BK-6) cells with minimal serum (stock culture approximately $10^{6.5-7}$ tissue culture infective dose ($TCID_{50}$/ml). A lysate of uninfected BK-6 cells was the tissue culture control. Results are reported delta counts per minute (cpm) that were determined as follows: [mean cpm in wells cultured with BK-6 cells infected with BRSV] - [mean cpm in wells cultured with uninfected BK-6 cells] and were used to compare groups of vaccinees at different time points. Lymphocyte blastogenic responses to Delta values were transformed (natural log) prior to statistical analysis (Hughes et al., 1984).

ELISA for interferon –γ- Interferon-γ (IFN–γ)was quantitated in virus-stimulated and control leukocyte cultures collected 60 hr after the initiation of culture (Ellis et al., 1992) using an IFN-γ-specific ELISA. Recombinant bovine interferon gamma was used to generate a standard curve and the levels of interferon gamma in test wells were determined by regression analysis using Microplate Manager® software (Biorad). The concentration of virus-specific interferon was determined as follows: (concentration of IFN in virus-stimulated wells) - (concentration of IFN in wells cultured with uninfected BK lysate).

Results are reported as picograms of IFN-γ.

Statistical analyses-Differences in responses between day 0 and day 12 and between the 2 groups of calves were evaluated using unpaired and paired students *t* -tests. Critical alpha level for significance of statistical tests was set at p=0.05. Analyses were performed using Statworksr software (Cricket Software, Philadelphia, PA).

Results

Antibody responses to BRSV and BHV-1 (Table 3)- Day 0 VN antibody titers to BRSV and BHV-1 were generally low (usually <6) in the calves. There were no significant differences between the groups of calves. Significant increases in serum antibodies were noted on day 12 following vaccination within 24 hr after weaning. In the calves that had not received perinatal immunizations, comparing day 0 to day 12 there was a significant increases in antibodies to both BRSV ($p<0.0001$) and BHV-1 ($p=0.0005$). Similarly in the calves that received perinatal immunizations there were significant increases in antibodies to both BRSV ($p<0.0001$) and BHV-1 ($p=0.0009$).

Lymphocyte blastogenic responses to BRSV and BHV-1 (Table 3)- Prior to the immunization at weaning, the calves that received perinatal vaccinations had significantly higher blastogenic responses to both BRSV ($p=0.009$) and BHV-1 ($p=0.03$) on day 0 than their penmates that did not receive immunizations during the first two weeks of life. Comparing day 0 and day 12, calves that had not been vaccinated in the perinatal period had significant increases in blastogenic responses to both BRSV ($p=0.035$) and BHV-1($p=0.0003$). Comparing day 0 and day 12, calves that had perinatal vaccinations had significant increases in blastogenic responses to BHV-1($p=0.003$). Comparing day 0 and day 12, there were no significant differences in BRSV-specific blastogenic responses after vaccination at weaning in the group calves that received immunizations during the first 2 weeks of life. Comparing lymphocyte blastogenic responses to BRSV and BHV-1 on day 12,there were no significant differences between the two groups of calves.

Interferon -γ in virus-stimulated leukocyte cultures (Table 3)- BRSV and BHV-stimulated leukocyte cultures from many calves had IFN-γ both before and after immunization at weaning. There was a high degree of variation in the levels of INF–γ in these cultures. No significant differences were found either between groups or between time points.

Discussion

1) There were low levels of BRSV and BHV-specific serum neutralizing antibodies in both groups of calves prior to immunization at weaning, with no significant differences between groups. One immunization with modified-live virus vaccine in these newly weaned "bawling" calves resulted in significant increases in serum neutralizing antibodies in both groups. The lack of significant

differences between groups indicated that there was no apparent enhanced response in the calves that received neonatal vaccinations.

2) Calves that were vaccinated during the first two weeks of life had significantly higher responses to BRSV and BHV at weaning compared to their unvaccinated penmates. One immunization with modified-live virus vaccine in recently weaned, previously unvaccinated calves induced significant levels of lymphocyte proliferation to both BRSV and BHV. The lack of significant differences between groups indicated that there was no apparent enhanced response in the calves that received neonatal vaccinations.

3) Secretion of INF-γ, as detected by the highly sensitive and highly specific ELISA was extremely variable in leukocyte cultures from vaccinated and unvaccinated calves, and after vaccination of both groups. The fact that IFN-γ was secreted by lymphocytes from calves with no apparent previous exposure to BRSV or BHV suggests that stimulation of macrophages by viral antigens and subsequent release of IL-12 may have been involved in the IFN-γ secretion rather than via the classical pathway involving "immune" T cells.

Conclusions and Impact

The major finding of this phase of the study was documentation that immunization with modified-live virus vaccine during the first two weeks of life results in the induction of long-lived memory T cell responses to both BRSV and BHV. This was evinced by significantly higher levels of lymphocyte proliferation in response to both viruses at weaning, approximately 5 months after the initial vaccination, in the calves that were vaccinated neonatally compared to their penmates that were not immunized in the neonatal period. Although there was no apparent enhanced immunity in calves that were immunized perinatally, the presence of memory T cells could be advantageous in defense against respiratory pathogens encountered in the period between neonatal vaccination and weaning. In addition, the stimulation of cellular and antibody responses to viral antigens in recently weaned calves suggests that this modified-live virus vaccine can be used effectively in animals that are undergoing the stress of weaning.

Table 3. Immune responses in weaned calves

Test/Time	Unvaccinated Calves[1]	Vaccinated Calves[2]
BRSV SN[3] / day 0 (weaning)	0.4 ± 0.2	0.1 ± 0.1
BRSV SN / day 12	6.1 ± 0.4	7.1 ± 0.3
BHV-1 SN / day 0	0.1 ± 0.1	0.2 ± 0.1
BHV-1 SN / day 12	1.2 ± 0.4	2.4 ± 0.5
BRSV LBT[4] / day 0	3.6 ± 0.7	6.2 ± 0.6*
BRSV LBT / day 12	5.8 ± 0.6	6.9 ± 0.6
BHV-1 LBT / day 0	1.8 ± 0.7	4.4 ± 0.9*
BHV-1 LBT / day 12	6.5 ± 0.7	7.4 ± 0.7
BRSV IFNg[5] / day 0	76.0 ± 17.0	85.4 ± 12.5
BRSV IFNγ / day 12	154.0 ± 38.8	130.0 ± 31.6
BHV-1 IFNγ / day 0	249.0 ± 51.0	248.0 ± 46.8
BHV-1 IFNγ / day 12	225.0 ± 66.1	210.0 ± 67.5

[1] Calves received no neonatal vaccination and one vaccination at weaning.

[2] Calves received one neonatal vaccination and one vaccination at weaning.

[3] Serum neutralizing antibodies. Data are presented as geometric mean titer ± standard error of the mean.

[4] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

[5] Interferon gamma. Quantitated with Enzyme-linked immunosorbant assay. Data are presented as group mean picograms / ml ± standard error of the mean.

* indicates significant ($p<0.05$) difference between groups

References

1) Durham PJK, Hassard LE. Prevalence of antibodies to infectious bovine rhinotracheitis, parainfluenza 3, bovine respiratory syncytial viruses in cattle in Saskatchewan and Alberta. *Can Vet J* 1990;31:815-820.

2) Ellis JA, Belden EL, Haven TR, Cavender J. Bovine respiratory syncytial virus-specific immune responses in cattle following immunization with modified-live and inactivated vaccines. Analysis of proliferation and synthesis of lymphokines by leukocytes in vitro. *Vet Immunol Immunopathol* 1992;34:21-34.

3) Ellis JA, Hassard LE, Morley PS. Comparative bovine respiratory syncytial virus-specific antibody and T lymphocyte responses in cattle following immunization with commercial polyvalent vaccines. *JAVMA* (submitted).

4) Ellis JA, Russell HI, Cavender J, Haven J. Bovine respiratory syncytial virus-specific immune responses in cattle following immunization with modified-live and inactivated vaccines. Analysis of specificity and activity of serum antibodies. *Vet Immunol Immunopathol* 1992;34:35-45.

5) Hughes HPA, Connelly CA, Strangeways M, Hudson L. Antigen specific lymphocyte transformation induced by secreted antigens from *Toxoplasma gondii*. *Clin Exptl Immunol* 1984; 58:539-547.

6) Kimman TG, Westenbrink F, Straver PJ. Priming for local and systemic antibody memory responses to bovine respiratory syncytial virus: effect and amount of virus, virus replication, route of administration and maternal antibodies. *Vet Immunol Immunopathol* 1989;22:145-160.

7) Scott R, Scott M, Toms GL. Cellular reactivity to respiratory syncytial virus in human colostrum and breast milk. *J Med Virol* 1895;17:83-89.

8) Sheldrake RF, Husband AJ. Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs. *Res Vet Sci* 1985;36:10-15.

9) Thrusfield M. Serological Epidemiology. In: *Veterinary Epidemiology*, Butterworths, Toronto. 1986;175-186.

10) Tizard I. Veterinary Immunology 4th ed. pp.257-259;261-276, W.B. Saunders, Toronto, 1992.

c) Presentations at Scientific Meetings and Manuscripts

1) Data derived from this study was presented at the following meetings:

a) *"How Can We Measure a Relevant Immune Response?"* presented at : The Annual Meeting of the American Association of Bovine Practitioners, Albuquerque, New Mexico, September, 1993

b) *" Immune Responses to Bovine Respiratory Syncytial*

Virus after Infection and Immunization"
presented at: The Ecole Nationale Veterinaire d' Alfort, Paris, France, September, 1993

c) *"Immune Responses to Bovine Respiratory Syncytial Virus after Infection and Immunization"*
presented at: The Bovine Respiratory Disease Symposium, Munich, Germany, September, 1993

2) A manuscript entitled, "The effect of perinatal vaccination on humoral and cellular immune responses in cows and young calves," has been prepared and submitted to the *Journal of the American Veterinary Medical Association* (see appended copy).

e) Personnel

	role in project	%time	total wages paid by ADF
John Ellis	prinicipal investigator	10	none
Lori Hassard	technician	10	none
Jaret Bogdan	technician	10	$1969.80

f) Equipment

none purchased

The Effect of Perinatal Vaccination on Humoral and Cellular Immune Responses in Cows and Young Calves

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Summary

A trial was undertaken to determine the effect of perinatal vaccination on immune responses in cows and calves. Fifty two cows were randomly assigned to two groups. One group of 26 cows was vaccinated twice with a polyvalent vaccine approximately 28 and 14 days prior to calving. The other group of 26 cows served as unvaccinated commingled controls. Thirty three calves that were born within approximately 2 weeks were used as principals in the first part of this study; thirteen calves from vaccinated dams and 12 from unvaccinated dams. An additional 8 calves from unvaccinated dams served as unvaccinated commingled controls. Sera were collected from cows on the day of vaccination and at calving. Colostrum was collected within 24 hours of calving. Antibodies and lymphocyte proliferative responses to BRSV and BHV-1 were measured in blood and colostrum. In addition, calves were assessed for passive transfer of lymphocytes that mediate delayed type hypersensitivity (DTH) responses to BRSV at 8 days of age prior to immunization. Lymphocyte proliferative responses and antibody production were again assessed in response to vaccination at weaning in a group of 16 neonatally vaccinated calves and an additional 15 unvaccinated penmates.

On the basis of enzyme-linked immunosorbent assays, all the multiparous cows used in this study had serological evidence of previous exposure to BRSV and BHV-1 and there were no significant differences between the groups prior to immunization. At parturition there were significant increases in serum antibodies to BRSV and BHV-1 in immunized cows. There were no significant differences in the amounts of BRSV-specific or BHV-1 specific antibodies in the colostrum between groups of cows. Calves from immunized cows had significantly higher concentrations of BRSV-specific serum antibodies on days 10 and 22 of life compared to commingled calves from unimmunized dams; however, there were no significant differences in serum antibodies specific for BHV-1 between the two groups of calves. There were no differences in the concentrations of virus-

specific antibodies either prior to or after vaccination at weaning between groups of neonatally vaccinated and unvaccinated calves.

Blood lymphocytes from vaccinated cows had significantly higher blastogenic responses to BRSV than cells from their non-immunized penmates. Colostral leukocyte populations from both groups of cows generally contained a high percentage of macrophages and had poor proliferative responses to BRSV antigens. Although blood leukocytes from young calves had low or suppressed responses to live BRSV at 10 days of age, immunization at this time induced significant increases in blastogenic responses to BRSV and BHV. Prior to vaccination at weaning, lymphocytes from neonatally vaccinated calves had significantly higher levels virus-specific proliferation than unvaccinated penmates. There were no differences between groups after vaccination at weaning. Leukocytes from most vaccinated and unvaccinated calves produced interferon gamma upon exposure to BHV-1 or BRSV in vitro and there were no differences among treatment groups.

Our results indicate that although there were usually no measurable increases in humoral responses to modified-live viruses following vaccination of young calves there was virus-specific proliferation of lymphocytes from most vaccinated calves. Virus-specific memory cells persisted in most calves until weaning. These findings suggest that the cellular immune system of young calves with maternal antibodies can be primed by immunization with modified-live viruses during the neonatal period.

Introduction

Except in cases of intrauterine infection, when a calf is born it has very little circulating immunoglobulin and, therefore, must rely on the antibody transferred from colostrum for protection against pathogens.[1] Partial or complete failure of passive transfer of colostral immunoglobulins is a primary cause of neonatal disease and mortality in calves.[2,3] Calves with inadequate IgG are four

times as likely to die and twice as likely to suffer disease as calves with an adequate concentration of circulating IgG.[3] There has been an increasing interest in the vaccination of young calves as a means of supplementing the protective effects of maternally derived antibodies. There are reports of decreased morbidity and mortality in calves that are vaccinated in the first weeks of life (S. McGuirk et al., unpublished data); however, few studies are reported and mechanisms involved in mediating the observed disease-sparing effects are poorly understood. According to current dogma [1,4] vaccination of young animals with maternal antibody will not be successful in stimulating immunity, due to the blocking effect that these antibodies have on vaccinal antigens.

The role of colostral antibodies in protection of young calves from bovine respiratory syncytial virus (BRSV) and bovine herpes virus-1 (BHV-) is controversial.[5] Previously it was thought that colostral antibodies to BRSV were not protective and would only block active immunization.[5] Recently, it has been shown that passively transferred colostral antibodies lessen the severity of disease following BRSV infection of young calves.[7] Furthermore, Kimman and colleagues[8] have documented that calves with maternal antibodies can be primed to have enhanced mucosal and serum antibody responses to BRSV infection by intranasal immunization with live BRSV. Most data indicate the primary importance cellular immunity in the recovery from BHV-1 infections, but there is little data on immunity to BHV-1 in the young calf.[6]

Currently little is known concerning cellular immune responses in the young calf, the role of these responses in viral infections, and how they may be enhanced by vaccination of young animals. Available data stress the role of colostral antibodies, but tend to overlook the potential role of macrophages and T and B lymphocytes that are also present in colostrum, in addition to antibodies, and can be passively transferred into the systemic circulation of the calf.[1,9] Moreover, little is know regarding how the reportedly suppressed immune system of the periparturient cow[10] responds to perinatal vaccination.

The purpose of this study was fourfold: 1) to examine the effect of

perinatal immunization on the stimulation of cellular and antibody responses in cows; 2) to determine the effects of maternal vaccination on passive transfer of antibody and cells to the calf; 3) to assess the role of maternal antibodies in modulating the effects neonatal vaccination in stimulating the immune system of young calves; and 4) to determine if neonatal vaccination effected immune responses to vaccine administered to calves at weaning.

Materials and Methods

Experimental Design- This study was conducted in the beef cattle herd at the University of Saskatchewan. Pregnant dams in this herd are routinely annually immunized against BHV-1 using a modified live vaccine[a] that is administered in the fall prior to spring calving. Cows are not routinely immunized against BRSV.

This study was conducted in two phases. In phase 1, in order ensure that at least 32 calves would be born over a period of approximately two weeks, fifty-two cows were randomly assigned to two groups. One group of 26 were vaccinated twice with a polyvalent vaccine[b] containing modified-live BRSV, BHV-1, parainfluenza-3 and inactivated bovine viral diarrhea virus approximately 28 and 14 days prior to calving. At these times the dams also received a bacterin[c] containing *Pasteurella hemolytica*.. The other group of 26 cows served as unvaccinated commingled controls. Twenty-four calves, 13 from immunized dams and 11 from unimmunized dams, were vaccinated with both vaccines on day 9 after birth. Another group of 8 unvaccinated calves from dams that were not vaccinated served as commingled controls.

Sera were collected from the cows on each day of vaccination and at calving. Antibodies to BRSV and BHV-1 were assessed using enzyme-linked immunosorbent assays (ELISA) and serum neutralization (SN). Colostrum was collected from the cows within 24 hr after calving. Antibodies and lymphocyte blastogenic responses to BRSV and BHV-1 were assessed in calves using blood

collected on the day of vaccination and 12 days after vaccination. Cellular immune responses were assessed in the peripheral blood of cows and calves at the time of parturition, and in colostrum. All assays were conducted in blind fashion. Cows and calves were observed daily by the herdsmen to monitor for adverse clinical signs.

In phase 2 of the study, calves were weaned at approximately 6 months of age when they weighed approximately 400-500 lb. Weaned calves were kept commingled in two adjoining pens and were separated from other cattle throughout the remainder of the study. One group of 16 calves had been immunized neonatally; the other group of 15 control calves had not received perinatal immunizations. All calves were vaccinated with both the polyvalent viral vaccine and the *Pastuerella* bacterin on the day after weaning.

Serum was collected from the calves on day of vaccination at weaning (weaning day 0) and on day 12 after immunization (weaning day 12). Serum samples were aliquoted, frozen and stored until antibodies to BRSV and BHV-1 were quantitated in one set of assays using serum neutralization. Cellular immune responses were assessed by lymphocyte blastogenesis and IFN-$_g$ production using leukocytes collected on weaning days 0 and 12.

Enzyme-Linked Immunosorbent Assays (ELISA) -ELISAs for BRSV and BHV-1 antibodies utilized partially purified viruses derived from lysed and ultracentrifuged bovine tracheal cells infected with the BRSV or BHV-1 and was performed according to previously described methods[11] using duplicate serum samples diluted 1/50. Lysed ultrcentrifuged uninfected bovine tracheal cells were used as control antigen. The optical density values were converted to units that were determined by comparison of raw optical density values with standard curves that was generated by linear regression of results obtained with serial dilutions of serum from naturally exposed steers with high concentrations of antibodies to BRSV and BHV-1. If the antibody concentration was approximated as a negative value from the regression line, then the concentration was

considered to be 0.

Virus neutralization (VN) assays- Virus neutralizing antibodies in serum were measured using microtiter plaque reduction assay that was performed essentially according to previously described methods[12] using the 375 isolate of BRSV and a field isolate of BHV-1. Samples were tested in duplicate and serum was diluted using 3-fold serial dilutions (1:3-1:81). Samples were further diluted 1:1 with cultured virus so that the reciprocal dilutions tested were 6, 18, 54, and 162. Titers were recorded as the last dilution that yielded positive results (neutralization of infectivity) in both replicates. If only one replicate was positive, it was considered to have one-half titer lower concentration. For the purpose of analyses, VN reciprocal titers were coded; titers recorded as <6, 6, 12, 18, 36, 54, 108, 162, >162, were assigned codes of 0, 1, 2, 3, 4, 5, 6, 7, or 8 respectively.[13]

Lymphocyte Blastogenesis Assays- Jugular blood was collected from vaccinated and unvaccinated cows at the time of calving, and from calves prior to vaccination at 10 days of age and on day 12 after vaccination. Mononuclear leukocytes were separated from the blood and colostrum using density gradient centrifugation,[14,15] with Ficoll/Hypaque of specific gravities 1.077 and 1.083, respectively. Lymphocyte blastogenesis assays were performed according to previously described methods[14] using a standardized dilution of live BRSV 375 isolate that was grown in bovine kidney (BK-6) cells with minimal serum (stock culture approximately $10^{6.5-7}$ tissue culture infective dose $(TCID)_{50}$/ml) or BHV-1 that was grown in Maden-Darby bovine kidney (MDBK) cells (stock culture approximately 10^{8} tissue culture infective dose $(TCID)_{50}$/ml). In order to abrogate the suppressive effect of BHV-1 on proliferation of cultured leukocytes,[16] the BHV-1 antigen was UV-inactivated and heat treated (56 C for 30 minutes). Lysates of uninfected BK-6 or MDBK cells were the tissue culture controls in the blastogenesis assays for BRSV and BHV-1, respectively. Standardized dilutions of antigens were determined as previously described.[14] Results are reported delta counts per minute (cpm) that were determined as

follows: [mean cpm in wells cultured with BK-6 cells or MDBK cells infected with BRSV or BHV-1] - [mean cpm in wells cultured with uninfected BK-6 cells or MDBK]. Negative values were considered to be 0. Delta cpm data were used to compare groups of vaccinees at different time points and among treatment groups following log transformation.[17] As a positive control for lymphocyte proliferation, in some experiments triplicate cultures of leukocytes were also stimulated with the phytomitogen, concanavalin A (Con A; 5 mg/ml).

Phenotyping of Colostral Leukocytes- Cytospins were prepared from colostral leukocyte suspensions that were obtained by density gradient centrifugation.[14] Leukocytes were reacted with monoclonal antibodies specific for BoCD5, BoCD4, BoCD8 and BoCD11b (ILA-15) using an indirect immunoperoxidase method as previously described.[14,18] Colostral leukocytes from 6 cows were examined.

ELISA for Interferon gamma (IFN-γ) - An ELISA for bovine IFN-γ was performed using bovine IFN-γ-specific monoclonal and monospecific polyclonal (rabbit) antibodies as capture and detection reagents, respectively (D. Godson, unpublished data). IFN-γ was quantitated in supernatants from leukocyte cultures that were stimulated with BRSV, BHV-1 or Con A and incubated for 60 hr.[19] Recombinant bovine interferon gamma was used to generate a standard curve and the levels of IFN-γ in test wells were determined by regression analysis using Microplate Manager® software[d]. Concentrations of virus-specific interferon were calculated as follows: (concentration of IFN in virus-stimulated cultures) - (concentration of IFN in cultures with uninfected cell lysates). Results are reported as picogram/ml of IFN-γ.

Delayed Type Hypersensitivity (DTH) Reactions - As a further *in vivo* assessment of the passive transfer of cellular immunity to BRSV, cutaneous DTH tests similar to those used in the assessment of tuberculin responses were performed.[20] This was accomplished by injecting 100 ml of BK cell lysate (control) or BRSV-infected BK lysate (vaccine virus used in blastogenesis assays) intradermally in shaved skin of right and left cervical areas, respectively, of

calves on day 8 of age. Skin thickness was assessed at 24 hr and measured in both sites with a caliper 48 hr later.

Statistical Analyses- Responses in the treatment groups were analyzed for the antibody and IFN-γ concentrations and delta counts per minute values (following log transformation) for lymphocyte blastogenesis tests. Critical alpha level for significance of statistical tests was set at p=0.05. Differences between groups at time 1 were assessed using a two-sample *t* -test. Change in response between time 1 and time 2 was evaluated using a paired *t* -test. If significant change was noted, differences between groups at time 2 were assessed using a two sample *t* - test. Calf lymphocyte blastogenesis responses on day 22 were compared using ANOVA. If the model was significant, groups means were compared using Bonferonni's *t* -test for multiple comparisons. Least squares linear regression was used to determine if concentrations of serum antibody determined concentrations of colostral antibodies. Analyses were performed using Statworksr software[e] and SAS software[f].

Results

Clinical responses to immunization in cows and calves-No adverse effects were noted in pregnant cows or calves that received intramuscular injections of the polyvalent modified-live vaccine.

BRSV-Specific Antibody Responses in Cows- On the basis of ELISA all the multiparous cows used in this study had serological evidence of previous natural exposure to BRSV (ELISA units>20) prior to vaccination (Table 1), and there were no significant differences between groups in serum concentrations of BRSV-specific antibodies prior to inoculation. There were no significant increases in BRSV-specific antibodies when sera were compared before and after two immunizations using ELISA. Neither were there any significant changes in BRSV-specific antibodies in the commingled control cows that were not immunized (Table 1). However, there were significant ($p<0.003$) increases in SN

antibodies in vaccinated cows (Table 1). Although colostrum from all cows tested contained BRSV-specific antibodies, there were no significant differences between vaccinated and unvaccinated cows (Table 1). There was considerable variation noted at the time of periparturient milking in the degree of glandular filling due to suckling. Approximately 28% of the variation in the levels of BRSV-specific colostral antibody was predicted by variation in post partum serum concentrations of BRSV-specific antibody as determined by ELISA ($p=0.003$; $R^2=0.273$).

BHV-1-Specific Antibody Responses in Cows- All cows used in this study were previously vaccinated with modified-live BHV-1 early in the fall prior to calving the following spring, and on the basis of ELISA all these multiparous cows had antibody responses to BHV-1 (ELISA units>20) prior to perinatal vaccination immunization (Table 1). There were no significant differences between groups in serum concentrations of BHV-specific antibodies prior to inoculation. There were significant ($p<0.001$) increases in BHV-1-specific antibodies in cows that received 2 immunizations, and no significant changes in BHV-1-specific antibodies in the commingled control cows that were not immunized (Table 1). After immunization, serum concentrations of BHV-1-specific antibodies were significantly higher in vaccinated cows compared to unvaccinated penmates. Although colostrum from all cows tested contained BHV-1-specific antibodies, there were no significant differences between vaccinated and unvaccinated cows (Table 1). Approximately 35% of the variation in the levels of BHV-1-specific colostral antibody was predicted by variation in post partum serum concentrations of BHV-1-specific antibody as determined by ELISA ($p<0.0001$; $R^2=0.354$).

BRSV- and BHV-1-Specific Lymphocyte proliferative Responses in Cows - Cows that were immunized twice in the late stages of pregnancy had significantly ($p=0.013$) higher blastogenic to BRSV than unvaccinated penmates when peripheral blood leukocytes were tested post partum (Table 1). Leukocytes from

several randomly selected cows also had proliferative responses to BHV-1 (data not shown).

Cellular responses in colostrum - In 5 of 6 randomly sampled colostrum samples ILA-15+ macrophages were the predominate population and accounted for 50-90% of cells, as determined on the basis of morphological and immunohistochemical criteria (Figure 1). CD5+ were a minority population in colostral samples examined and comprised approximately equal numbers of CD5+/CD4+ and CD5+/CD8+ lymphocytes (Figure 2). Colostral leukocyte populations had poor (delta cpm values <200) proliferative responses to BRSV antigens (data not shown).

BRSV-Specific Antibody Responses in Neonatal Calves- Calves from vaccinated cows had significantly higher concentrations of BRSV-specific antibodies in there serum at day 10 ($p=0.008$) and day 22 ($p=0.038$) of age (Table 2). There was a significant decrease in the BRSV-specific serum antibodies in the calves from vaccinated cows, comparing day 10 and day 22 (12 days after immunization) and no significant change in the calves from unvaccinated cows (Table 2).

BHV-1-Specific Antibody Responses in Neonatal Calves- All calves tested had evidence of passive transfer of BHV-1 specific antibodies; however, there were no significant differences in BHV-1-specific antibodies in the serum of calves from vaccinated or unvaccinated cows on day 10 or day 22 of life (Table 2) and both groups had signifcant decreases in BHV-1 specific antibodies between days 10 and 22.

Antibody responses to BRSV and BHV-1 in Weaned Calves- VN antibody titers to BRSV and BHV-1 were generally low (usually <6) in the calves on the day after weaning. There were no significant differences between the groups of calves. Significant increases in serum antibodies were noted on weaning day 12 following vaccination (within 24 hr after weaning). In the calves that had not received perinatal immunizations, comparing weaning day 0 to weaning day 12 there were significant increases in antibodies to both BRSV ($p<0.0001$) and BHV-

1 (p=0.0005). Similarly in the calves that received perinatal immunizations there were significant increases in antibodies to both BRSV (p<0.0001) and BHV-1 (p=0.0009; Table 2); however, there were no significant differences between groups.

Lymphocyte Proliferative Responses to BRSV, BHV-1, or Mitogen in Calves- Leukocytes from 10-day old calves harvested prior to immunization had poor and often suppressed responses to BRSV and BHV-1, irrespective of the vaccination status of the dam (Table 2). Leukocytes from most 22-day old calves harvested 12 days after one immunization with the polyvalent vaccine responded to BRSV and BHV-1 (Figure 2). The responses BRSV were significantly (p<0.001) higher than responses on day 10 (Table 2). There were significantly higher proliferative responses to BRSV (p=0.015) and BHV-1 (p=0.006) in vaccinated calves compared to responses in a group of age-matched unvaccinated calves from unvaccinated dams. There were no significant differences in lymphocyte proliferative responses to BRSV or BHV-1 between groups of vaccinated calves from vaccinated and unvaccinated dams. Leukocytes from 22-day old calves had variable proliferative responses to concanavalin-A (Table 2). There were no significant differences in proliferative responses to the mitogen among the groups of vaccinated calves from vaccinated and unvaccinated dams and unvaccinated calves.

Prior to the immunization at weaning, the calves that received perinatal vaccinations had significantly higher blastogenic responses to both BRSV (p=0.009) and BHV-1 (p=0.03) on weaning day 0 than their penmates that did not receive immunizations during the first two weeks of life (Table 3). Comparing weaning day 0 and weaning day 12, calves that had not been vaccinated in the perinatal period had significant increases in blastogenic responses to both BRSV (p=0.035) and BHV-1(p=0.0003). Comparing weaning day 0 and weaning day 12, calves that had perinatal vaccinations had significant increases in blastogenic responses to BHV-1(p=0.003). Comparing weaning day 0 and weaning day 12, there were no significant differences in BRSV-specific blastogenic responses after

vaccination at weaning in the group calves that received immunizations during the first 2 weeks of life. Comparing lymphocyte blastogenic responses to BRSV and BHV-1 on weaning day 12,there were no significant differences between the two groups of calves.

Interferon-Gamma (IFN-γ) Responses to BRSV, BHV or Mitogen in Calves - There was IFN-γ in the supernatants from leukocyte cultures stimulated with BRSV or BHV-1 from most calves tested at 22 days of age (Table 2). Levels of this cytokine produced in response to these antigens were markedly lower than those produced in response to the mitogen concanavalin-A (Table 2). In contrast to lymphocyte blastogenic responses, there were no significant differences between groups of vaccinated and unvaccinated neonatal calves.

BRSV and BHV-stimulated leukocyte cultures from many weaned calves had IFN -γ both before and after immunization at weaning (Table 3). There was a high degree of variation in the levels of INF-γ in these cultures. No significant differences were found either between groups or between time points.

Delayed - Type Hypersensitivity (DTH) Reactions to BRSV in Neonatal Calves - No measurable or palpable DTH reactions were observed following intradermal inoculation of 8 day old calves with modified live BRSV. Neither were there any reactions at the dermal sites of inoculation uninfected culture supernatants (controls).

Discussion

There have been relatively few studies of immune responses to vaccines in pregnant cattle. Available data is conflicting with regard to the role of periparturient immunosuppression in modulating cellular and antibody responses in the cow.[10,21] Our results are consistent with a previous study[21] and provide further evidence that vaccines containing modifed-live viruses can stimulate T lymphocyte and serum antibody responses in seropositive pregnant cattle. There is a dearth of knowledge concerning vaccine-induced immune responses in young

calves, especially with regard to stimulation of cellular immunity. Our findings indicate that young calves can respond to vaccination early in life and suggest that the immune system of the neonate can be primed in the presence of circulating maternal antibodies.

Although immunization of BRSV-seropositive cattle with this polyvalent vaccine in the late stages of pregnancy did not result in significant increases in BRSV-specific antibodies in the serum, as measured by ELISA, there were significant increases in serum neutralizing antibodies. We have previously reported a disparity in results between ELISA and SN assays for BRSV antibodies in vaccinated cattle.[11] These findings are consistent with the observation that this modified-live BRSV virus apparently does not induce high levels of ELISA-reactive antibody relative to neutralizing antibody.[11] Evidence of vaccine-induced production of BRSV antibodies in seropositive cows complements other reports[21] documenting that immunization of seronegative pregnant cattle with live viruses given in adjuvant can result in a significant increase in serum antibodies. Similar to BRSV SN antibodies, administration of this polyvalent vaccine in the late stages of pregnancy stimulated significant increases BHV-1-specific serum antibodies, as measured by ELISA. Although the concentration of BHV-1-specific SN antibodies was not determined, we have previously found a good correlation between results of ELISA and SN tests for BHV-1-specific antibodies (J. Ellis and L. Hassard, unpublished observations).

There were no significant differences between BRSV and BHV-specific antibodies in the colostrum of vaccinated and unvaccinated cows, as measured by ELISA. This may be in part attributable to the already high concentrations of antibodies to these viruses in the serum of the dams prior to immunization. In other words, there may have been maximal transfer of these specific antibodies from the serum to the colostrum. Alternatively, the high degree of variation in colostral samples due to collection of milk from quarters that had been suckled, or variation in the relative concentrations of antibodies in milk from quarters with subclinical inflammation may have precluded the statistical significance of

any differences. However, calves from dams vaccinated in the late stages of pregnancy did have significantly higher levels of BRSV-specific antibodies than calves from previously exposed dams that were not vaccinated in the late stages of pregnancy. This was in contrast to the lack of significant differences in BHV-specific serum antibodies in the calves. The reasons for the apparent disparity in BRSV versus BHV-specific serum antibodies in the calves are not apparent.

We obtained consistently no or low levels of virus-specific lymphocyte proliferative responses in populations of colostral leukocytes. These results are similar to those reported in previous studies[22] and could be indicative of the hyporesponsiveness of milk lymphocytes to mitogenic and antigenic stimulation when compared to blood lymphocytes. In contrast Scott, and colleagues[23] reported lymphocyte proliferative responses to human respiratory syncytial virus in human colostrum and breast milk. One major factor determining why we were unable to detect proliferation by colostral lymphocytes that were capable of clonal expansion[24] was the high proportion of potentially suppressive macrophages in our samples. As herein, previous studies have documented the preponderance of macrophages in periparturient milk and a minority of, predominately T, lymphocytes.[15] Depletion of the majority of the macrophages may be required in order to allow detectable lymphocyte proliferation.

These studies were unable to document passive transfer of cellular immunity to calves measurable by the proliferation of blood leukocytes taken from calves after suckling and prior to vaccination, nor by evidence of passive transfer of lymphocytes mediating BRSV-specific DTH responses. The fact that tuberculin responses can be transferred to newborn calves and human beings by intestinal absorption of maternal lymphocytes indicates that these cells probably play a role in the development of systemic immune responses to a variety of pathogens.[1] Moreover, Reidel-Caspari[25] demonstrated that following an experimental *Escherichia coli* infection calves that had received colostrum depleted of leukocytes excreted significantly more bacteria and had significantly

lower serum concentrations of IgA and IgM than calves that had ingested colostrum containing leukocytes. More sensitive methods may be necessary to detect the probably low frequency of maternal virus-specific T lymphocytes that are transferred to the neonate. Alternatively, this vaccine may not stimulate those subpopulations of T lymphocytes that would mediate delayed type hypersensitivity reactions to BRSV in cattle. Recent work in models of respiratory syncytial virus infections in laboratory rodents suggests that pulmonary DTH reactions are responsible for pathology and disease in RSV infections.[26] If this is also a pathologic mechanism in cattle, then it could be advantageous to design and use vaccines that do not induce DTH reactions.

The fact that immunization of young calves with this modified-live vaccine on day 10 of life did not result in the production of significantly increased serum levels of antibodies to BRSV or BHV-1 is compatible with the current dogma regarding the blocking effect of maternal antibodies on antibody response to modified-live viral vaccines.[1] However, the fact that some calves had slight increases in virus-specific serum antibodies, and many did not have the expected decrease in antibody that would occur in 12 days, which is more than 1/2 of the approximately 20 day 1/2 life of bovine IgG,[27] suggests that there was some stimulation of antibody production by the modified-live viruses contained in the vaccines.

Although antibody responses to BRSV and BHV-1 were generally not appreciable in the vaccinated neonatal calves, lymphocyte proliferative responses to the two viruses were significantly greater in vaccinated calves compared to unvaccinated penmates. This was apparently not effected by the vaccine status of the dam. The ability of perinatal immunization to stimulate virus-specific T lymphocyte responses in young calves provides evidence that "priming" of the cellular immune system can occur following parenteral delivery of modified live viruses despite the presence of maternal antibodies. Kimman et al.[7] have previously shown that priming of the immune system for anamnestic antibody responses can take place in the presence of maternal antibodies if live BRSV is

given via a mucosal route. Our data suggest that T cells are primed and can mount a secondary response upon exposure to antigen (*in vitro)* in the absence of significant antibody responses. Furthermore, the fact that neonatally vaccinated calves had significantly higher virus-specific lymphocyte blastogenic responses prior to vaccination at weaning than unvaccinated penmates, indicates that neonatal immunization can induce long-lived memory T cell responses. These data may reflect a difference between the neonatal cellular responses to BRSV in calves and to experimental human respiratory syncytial virus (HRSV) in young rodents.[28] Passive transfer of monoclonal antibodies of the IgG1 isotype with specificity for the fusion (F) and glycoprotein (G) of HRSV are reported to inhibit RSV-specific secondary cytotoxic T cells responses in neonatal mice.[28] Further studies are required to determine the types of T cells that respond to viral antigens in young calves.

The ability of the neonatal and weaned cattle to respond to viral antigens with IFN-g production is a further measure of cellular immunity. The lack of significant differences in IFN-γ production among the various treatment groups suggests that the cells producing this cytokine in culture were not clonally expanded memory T lymphocytes, but rather natural killer cells or some other cells that were not dependent on expansion of virus-specific clones. The apparent lack of anamnestic IFN-γ production by cells from vaccinated calves may indicate that the viral antigens interacted with macrophages in cultures and stimulated IFN production through an IL-12-mediated pathway.[29]

The levels of lymphoid proliferation in response to viral antigens and Con A, and the levels of IFN-γ production in these young calves were generally lower than those observed in weanling or adult cattle. Low levels of lymphoid proliferation in response to mitogens as observed here have been previously reported[30,31] and could be a function of the immaturity of immune cell function or the predominance of $\gamma\delta$ T lymphocytes in these young calves. The latter T cells that comprise the majority of the large "null" cell population in calves are less responses to mitogens and antigens than the ab T cells that predominate in the

blood of older cattle.[32]

In conclusion, the major finding of this study is documentation of stimulation of virus-specific T lymphocytes following neonatal inoculation of young calves with vaccines containing modified-live viruses. These T cells comprise a population of cells with virus-specific immunological memory that persist until at least weaning in the neonatally vaccinated calves. This is an indication that stimulation of cell-mediated immune responses can occur in the presence of maternal antibody. Such evidence of priming of T cells by immunization of neonates may, in part, explain the reported reduced clinical disease following subsequent exposure of young vaccinees to viral infections.

References

1) Tizard I. Veterinary Immunology 4th ed. Toronto, Canada, W.B. Saunders,1992;257-259 , 261-276,

2 Banks KL, McGuire TC. Neonatal immulogy. In: Haliwell REW, Gorman NT, eds. Veterinary clinical immunology. Philadelphia,W.B. Saunders, 1989;197-202.

3. White DG, Andrews AH: Adequate concentration of circulating colostral proteins for market calves. *Vet Rec* 1986;119:112-113.

4. Husband AJ, Lascelles AK. Antibody responses to neonatal immunisation in calves. *Res Vet Sci* 1975;18:201-207.

5. Stott EJ, Taylor G. Respiratory syncytial virus:brief review. *Arch Virol* 1985;84:1-52.

6) Rouse BT, Babiuk LA. Mechanisms of recovery from herpesvirus

infections - review. *Can J Comp Med* 1978;42:414-427.

7) Belknap EB, Baker JC, Patterson JS, Walker RD, Haines DM, Clark EG. The role of passive immunity in bovine respiratory syncytial virus-infected calves. *J Infect Dis* 1991;163:470-476.

8) Kimman TG, Westenbrink F, Straver PJ. Priming for local and systemic antibody memory responses to bovine respiratory syncytial virus: effect and amount of virus, virus replication, route of administration and maternal antibodies. *Vet Immunol Immunopathol* 1989;22:145-160.

9) Sheldrake RF, Husband AJ. Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs. *Res Vet Sci* 1985;36:10-15.

10) Kehrli ME Jr, Nonnecke BJ, Roth JA. Alterations in bovine lymphocyte function during the periparturient period. *Am J Vet Res* 1989;50:215-220.

11) 3) Ellis JA, Hassard LE, Morley PS. Comparative bovine respiratory syncytial virus-specific antibody and T lymphocyte responses in cattle following immunization with commercial polyvalent vaccines. *J AmVet Med Assoc* (in press).

12) Ellis JA, Russell HI, Cavender J, Haven J. Bovine respiratory syncytial virus-specific immune responses in cattle following immunization with modified-live and inactivated vaccines. Analysis of specificity and activity of serum antibodies. *Vet Immunol Immunopathol* 1992;34:35-45.

13) Thrusfield M. Serological Epidemiology. In: *Veterinary Epidemiology*, Butterworths, Toronto. 1986;175-186.

14) Ellis JA, Belden EL, Haven TR, Cavender J. Bovine respiratory syncytial virus-specific immune responses in cattle following immunization with modified-live and inactivated vaccines. Analysis of proliferation and synthesis of lymphokines by leukocytes in vitro. *Vet Immunol Immunopathol* 1992;34:21-34.

15) Park YH, Fox LK, Hamilton MJ, Davis WC. Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. *J Dairy Sci* 1992;75:998-1006.

16) Hutchings DL, Campos M, Qualtiere L, Babiuk LA. Inhibition of antigen-induced and interleukin-2-induced proliferation of bovine peripheral blood leukocytes by inactivated bovine herpes virus-1. *J Virol* 1990;69:4136-4151.

17) Hughes HPA, Connelly CA, Strangeways M, Hudson L. Antigen specific lymphocyte transformation induced by secreted antigens from *Toxoplasma gondii. Clin Exptl Immunol* 1984; 58:539-547.

18) Howard CJ, Morrison WI. Leukocyte antigens of cattle, sheep and goats. *Vet Immunol Immunopathol* 1991;271-94.

19) Emery DL, Dufty JH, Wood PR. An analysis of cellular proliferation, and synthesis of lymphokines and specific antibody in vitro by leukocytes from immunized cattle. *Vet Immunol Immunopathol* 1988;18:67-80.

20) Woodard LF, Renshaw HW, Burger D. Cell-mediated immunity in neonatal calves: delayed type hypersensitivity and lymphocyte blastogenesis following immunization with a mycobacterial immunopotentiating glycolipid and tuberculoproteins of *Mycobacterium bovis. Am J Vet Res* 1980; 41:561-563.

21) Archambault D, Morin G, Elazhary Y, Roy RS, Joncas JH. Immune

response of pregnant heifers and cows to bovine rotavirus infection in newborn calves fed colostral antibodies or colostral lymphocytes. *Am J Vet Res* 1988;49:1084-1091.

22) Nonnecke BJ, Kehrli ME Jr. Isolation of mononuclear cells from bovine milk by continuous flow and density gradient centrifugation: response of cells to mitogens. *Am J Vet Res* 1985;46:1259-1263.

23) Scott R, Scott M, Toms GL. Cellular reactivity to respiratory syncytial virus in human colostrum and breast milk. *J Med Virol* 1895;17:83-89.

24) Nonnecke BJ, Elsken LA, Kehrli ME. Local and systemic immune response in the cow after intramammary vaccination during lactation. *Vet Immunol Immunopathol* 1986;11:31-44.

25) Riedel-Caspari G. The influence of colostral leukocytes on the course of an experimental *Escherichia coli* infection and serum antibodies in neonatal calves. *Vet Immunol Immunopathol* 1993;35:275-288.

26) Connors M, Kulkarni AB, Firestone CY, Holmes KL, Morse HC, Sotnikov AV, Murphy BR. Pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of CD4+ T cells. *J Virol* 1992;66:7444-7451.

27) Logan EF, Penhale WJ, Jones RA. Changes in serum immunoglobulin levels of colostrum-fed calves during the first 12 weeks postpartum. *Res Vet Sci* 1973;14:394-397.

28) Bangham CRM. Passively acquired antibodies to respiratory syncytial virus impair the secondary cytotoxic T- cell response in the neonatal mouse.

Immunology 1986;59:37-41.

29) Scott P. IL-12: Initiation cytokine for cell-mediated immunity. *Science* 1993;260:496-497.

30) Fossum C, Larsson B, Matsson P. Development of mononuclear cell subpopulations and their functions during calfhood. *J Vet Med (B)* 1986;33:518-527.

31) Griebel PJ, Schoonderwoerd M, Babiuk LA. Ontogeny of the immune response: Effect of protein energy malnutrition in neonatal calves. *Can J Vet Res* 1987;51:428-435.

32) Clevers H, MacHugh ND, Bensaid A, Dunlap S, Baldwin CL, Kaushal A, Iams K, Howard CJ, Morrison WI. Identification of a bovine surface antigen uniquely expressed on CD4-CD8- T cell receptor g/d T lymphocytes. *Eur J Immunol* 1990;20:809-817.

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Footnotes

a Bovishield, Smithkline Beecham Animal Health, Exton, PA

b Cattlemaster 4, Smithkline Beecham Animal Health, Exton, PA

c Oneshot, Smithkline Beecham Animal Health, Exton, PA

d Biorad, Richmond, CA

e Cricket Software, Philadelphia, PA

f SAS Institute, Cary, NC

Table 1. Immune responses in cows

Test/Time	Unvaccinated Cows	Vaccinated Cows
BRSV ELISA[1] day 0	76.5 ± 3.2	84.6 ± 5.4
BRSV ELISA / pp[2]	70.0 ± 4.0	84.7 ± 4.8
BRSV SN[3] / day 0	5.4 ± 0.4	5.7 ± 0.4
BRSV SN[1] / pp	5.7 ± 0.4	7.2 ± 0.2*
BHV-1 ELISA / serum / day 0	58.8 ± 5.2	63.6 ± 7.3
BHV-1 ELISA / serum / pp	54.4 ± 4.4	81.3 ± 24.6*
BRSV ELISA / colostrum	66.0 ± 6.0	65.0 ± 6.0
BHV-1 ELISA / colostrum	59.0 ± 7.4	60.2 ± 1.2
BRSV LBT[4] / pp	6.2 ± 1.0	8.9 ± 0.3*

[1] Enzyme-linked immunosorbant assay. Data are presented as group mean ELISA units ± standard error of the mean.

[2]post partum

[3] Serum neutralizing antibodies. Data are presented as geometric mean titer ± standard error of the mean.

[4] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

* indicates significant ($p<0.05$) difference between groups

Table 2. Immune responses in neonatal calves

Test/Time	Unvaccinated Calves (Unvaccinated Cows)	Vaccinated Calves (Unvaccinated Cows)	Vaccinated Calves (Vaccinated Cows)
BRSV ELISA[1] / day 10	ND[2]	74.8 ± 4.1	94.8 ± 5.6*
BRSV ELISA / day 22	ND	72.5 ± 4.7	88.0 ± 4.6*
BHV-1 ELISA / day 10	ND	60.2 ± 5.8	61.9 ± 7.4
BHV-1 ELISA / day 22	ND	45.3 ± 4.9	53.1 ± 6.5
BRSV LBT[3] / day 10	ND	2.5 ± 0.7	1.3 ± 0.7
BRSV LBT / day 22	0.7 ± 0.7*	5.8 ± 1.2	5.0 ± 1.1
BHV-1 LBT / day 22	0.8 ± 0.8*	5.9 ± 1.2	5.8 ± 1.1
Con A[4] LBT / day 22	11.5 ± 0.1	10.8 ± 0.2	11.3 ± 0.1
BRSV IFNγ[5] / day 22	107.0 ± 56.0	77.0 ± 37.2	32.5 ± 13.0
BHV-1 IFNγ / day 22	132.0 ± 49.0	108.0 ± 49.0	62.0 ± 18.0
Con A IFNγ / day 22	1430.0 ± 337.0	732.0 ± 233.0	1150.0 ± 277.0

[1] Enzyme-linked immunosorbant assay. Data are presented as group mean ELISA units ± standard error of the mean.

[2] Not done

[3] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

[4] Concanavalin A (phytomitogen)

[5] Interferon gamma. Quantitated with ELISA. Data are presented as group mean picograms / ml ± standard error of the mean.

* indicates significant ($p<0.05$) difference between groups

Table 3. Immune responses in weaned calves

Test/Time	Unvaccinated Calves[1]	Vaccinated Calves[2]
BRSV SN[3] / day 0 (weaning)	0.4 ± 0.2	0.1 ± 0.1
BRSV SN / day 12	6.1 ± 0.4	7.1 ± 0.3
BHV-1 SN / day 0	0.1 ± 0.1	0.2 ± 0.1
BHV-1 SN / day 12	1.2 ± 0.4	2.4 ± 0.5
BRSV LBT [4] / day 0	3.6 ± 0.7	6.2 ± 0.6*
BRSV LBT / day 12	5.8 ± 0.6	6.9 ± 0.6
BHV-1 LBT / day 0	1.8 ± 0.7	4.4 ± 0.9*
BHV-1 LBT / day 12	6.5 ± 0.7	7.4 ± 0.7
BRSV IFNg[5] / day 0	76.0 ± 17.0	85.4 ± 12.5
BRSV IFNγ / day 12	154.0 ± 38.8	130.0 ± 31.6
BHV-1 IFNγ / day 0	249.0 ± 51.0	248.0 ± 46.8
BHV-1 IFNγ / day 12	225.0 ± 66.1	210.0 ± 67.5

[1] Calves received no neonatal vaccination and one vaccination at weaning.

[2] Calves received one neonatal vaccination and one vaccination at weaning.

[3] Serum neutralizing antibodies. Data are presented as geometric mean titer ± standard error of the mean.

[4] Lymphocyte blastogenesis test . Data presented as geometric mean of transformed (ln) delta counts per minute ± standard error of the mean

[5] Interferon gamma. Quantitated with Enzyme-linked immunosorbant assay. Data are presented as group mean picograms / ml ± standard error of the mean.

* indicates significant ($p<0.05$) difference between groups

Figure Legends

Figure 1. Cytospin of colostral leukocytes from a representative cow. Note predominance of ILA-15+ macrophages (brown-stained cells). Arrow indicates unstained lymphocyte. Immunoperoxidase stain.

Figure 2. Cytospin of colostral leukocytes. Lymphocytes are stained brown following reaction with a monoclonal antibody for bovine CD5, a pan T cell marker. Immunoperoxidase stain.

Figure 3. Invididual calves' lymphocyte proliferative responses to BHV-1 and BRSV in culture. Bars represent the degree of virus-specific proliferation of lymphocytes harvested at 22 days of age, 12 days after a single vaccination.

Figure 1

Figure 2

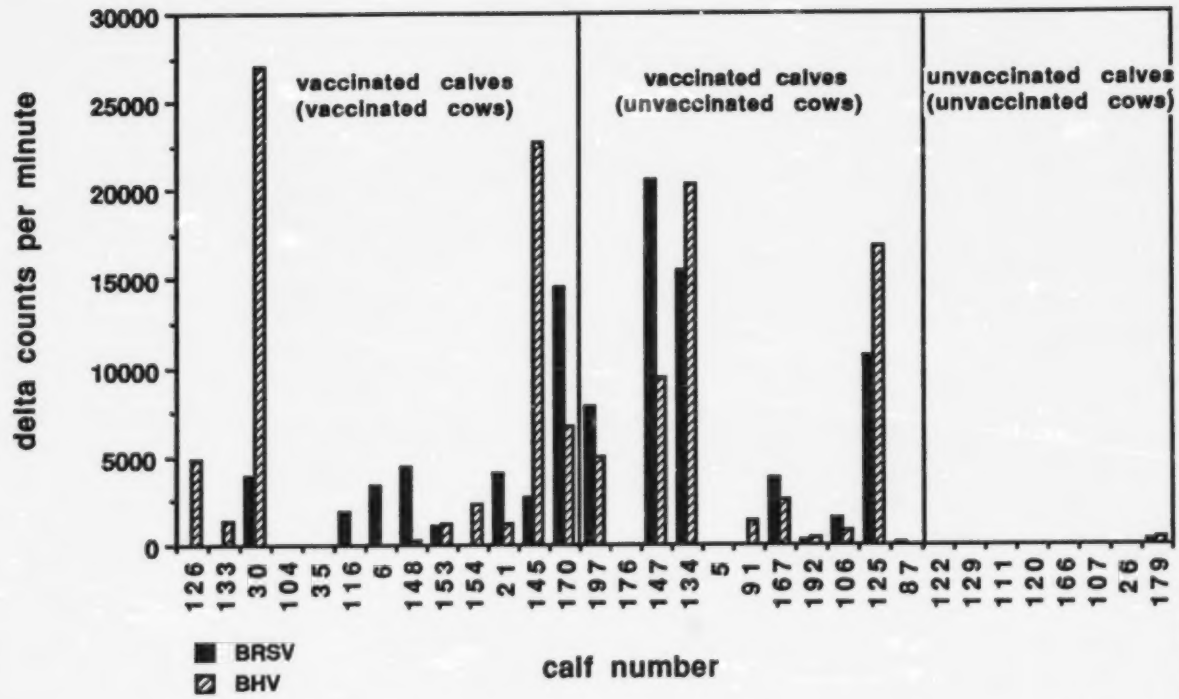
30000
25000
20000
15000
10000
5000
0
delta counts per minute
vaccinated calves
(vaccinated cows)
vaccinated calves
(unvaccinated cows)
unvaccinated calves
(unvaccinated cows)
126
133
30
104
35
116
6
148
153
154
21
145
170
197
176
147
134
5
91
167
192
106
125
87
122
129
111
120
166
107
26
179
BRSV
BHV
calf number

Figure 3